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Food Control 104 (2019) 50–56

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Ambient mass spectrometry based on REIMS for the rapid detection of adulteration of minced meats by the use of a range of additives

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ARTICLE INFO

Keywords:
Meat authentication
Rapid evaporative ionization mass spectrometry
Ambient mass spectrometry
Meat processing additives

ABSTRACT

Meat adulteration is a significant economic problem as it can result in substantial economic gains and loss of consumers' trust in the food industry. Addition of a bulking agent masking the addition of water into minced meat is a fraudulent practice that is very difficult to detect. The quality of the meat can be assessed by measurement of total net protein, however the methods used to measure such property are not able to cope with the quite sophisticated modern-day adulteration practices. In our study, we assessed the potential of recently introduced Rapid Evaporative Mass Spectrometry (REIMS) technology to discover undeclared additives in chopped pork and chicken meat-based products such as sausages and burgers. The REIMS technique was able to discover such adulterants with a high degree of confidence when more than 2.5% of these substances were added. The results could be obtained within a few minutes. In this context REIMS can be classified as a rapid screening method which could be employed as a front-line testing method to ensure the quality and authenticity of meat products.

1. Introduction

As with many food commodities, meat-based products are susceptible to fraud. Substitution by a cheaper meat, mislabelling or addition of undeclared constituents are the most common fraudulent practices (Abbas et al., 2018). In most cases, they are driven by the producer's financial profit and while they do not necessarily pose a risk to consumers' health such set out to cheat the consumer and businesses who work to the correct standards.

The quality of the meat can be determined by various methods but the procedure which correlates with quality is the determination of “net muscle protein” (NMP) which describes protein content in lean meat excluding protein in connective tissues. The NMP content is calculated as:

$$NMP = TPC - CC$$

where TPC is total protein content as measured by Kjeldahl method and CC is collagen content as calculated from 4-hydroxy-proline (4-OH-Pro) content measured by high performance liquid chromatography with spectrophotometric detector. Different tissues have different NMP content however, a higher protein content is an indicator of a better-quality meat.

This product characteristic however, may be not very informative when dealing with minced meat-based products such as sausages or burgers. The easiest way is to increase meat weight by water addition and, at the same time, to avoid the decrease of ´total nitrogen' content, some (cheap) nitrogen rich material can be added which means the standard Kjeldahl method will give a false protein score. If collagen is not used, the second part of the above equation defining ´net muscle protein' is not affected. This way, different proteins from plant sources (soy, beans) can be added to the meat product to substitute net muscle protein. Some of these additions, such as soy, are easily detectable by LC-MS/MS when typical markers, plant isoflavones such as daidzein and genistein, can be screened or by using a proteomic approach (Leitner, Castro-Rubio, Marina, & Lindner, 2006).

In addition, the outlined testing methods are both labour and time demanding, thus laboratory throughput is rather limited to support efficient control programmes. Thus, the availability of fast and reliable method enabling rapid screening of possibly adulterated samples is urgently needed. Any samples suspected of adulteration can be then examined by confirmatory methods such as polymerase chain reaction or liquid chromatography coupled to mass spectrometry which are

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https://doi.org/10.1016/j.foodcont.2018.10.029
Received 8 August 2018; Received in revised form 3 October 2018; Accepted 27 October 2018
Available online 30 October 2018

regularly employed to discover meat fraud.

In terms of screening method; ambient mass spectrometry (AMS) meets the requirements in terms of speed. Several AMS techniques have been developed and utilized for food fraud detection and have been reviewed recently.(Black, Chevallier, & Elliott, 2016), mainly desorption electrospray ionization (DESI) and direct analysis in real time (DART) which is based on ionization of analytes by metastable helium atoms and was thoroughly tested by our group (Hajslova, Cajka, & Vaclavik, 2011). More recently another AMS technique, Rapid Evaporative Ionization Mass Spectrometry (REIMS) developed by Takats (Takats, Denes, & Kinross, 2012) has been investigated for clinical analyses of intraoperative tissues. This technique relies on ionization and desorption of molecules by generating surgical smoke from a solid sample using an electrosurgical device. Since its discovery, REIMS has been employed in several food adulteration studies, namely for speciation of meat (Balog et al., 2016) and fish (Black et al., 2017). The separation of groups of samples was based on the profiles of anionic lipids. These were very specific for the animal species; however, they can also vary among different animals within the species. So far the studies focused on mislabelling of the origin of species or substitution of more expensive meat or fish with cheaper one. The matrices studied were either raw meat or coarsely ground burgers.

The objective of the present study was to develop a high-throughput method which allows for screening of protein-based meat additives in finely ground meat products such as sausages based on ambient ionization mass spectrometry. For this purpose, REIMS was the method of choice as it offers fast analysis time and no sample preparation.

2. Experimental

2.1. Chemicals and materials

2-propanol was supplied by Merck (Darmstadt, Germany), Leucine-enkephalin from Waters (USA). Carrageenan additive was Genugel MB-76F (CP Kelco, USA), Plasma powder, Protein S 80 and Protein Natur were kindly supplied by Department of Food Technology, UCT Prague. Scanpork D 80, Scanpork D 85 and Scanpork D 90 were purchased from Scanflavour (DK). Samples of collagen powder (designated as K4065, K4067 and K4565) were kindly supplied by State Veterinary Inspection, Prague.

The fine chicken breast samples were bought in local retail stores. Pork neck samples were either bought at the retail stores or kindly supplied by Dr. Rudolf Ševčík from Department of Food Conservation, UCT Prague and they were provided by trusted suppliers. When collected, the meat chunks were stored up to 3 days prior to further processing in a freezer at −20 °C.

2.2. Experiment design

To assess REIMS capability to detect addition of various bulking agents to meat either by detection of new, ´foreign' signals or as change in profiles of common meat signals, a series of experiments was designed. To simulate mixed meat products such as sausage or burger, five homogenates from various chicken breasts and pork necks were prepared and subsequently adulterated with protein powders. The adulterants PS80 protein powder, Naturprotein powder, pork plasma powder and carrageenan were added to one of the pork homogenates in weight ratios 0.5%, 1.0% and 2.5% (*i.e.* 12 samples). This homogenate was ´adulterated' by PS80 pork protein in concentrations ranging from 0.5 to 5%, w/w (with increments of 0.5) to assess how the samples with different and finer levels of adulteration, other than previously mentioned, behave within the model (7 samples). In addition 4 remaining homogenates were adulterated with PS80 protein on three previously mentioned (0.5, 1.0 and 2.5%) levels to assess intraspecies variability (12 samples). For repeatability assessment, replicates of one homogenate adulterated with 2.5% of PS80 powder were produced (5 samples). Then 2 replicates of the 5 homogenates were used as un-adulterated samples. In total 46 samples were prepared.

One of the chicken homogenates was ´adulterated' by three collagen powders as well with separate tissue (low grade material obtained by separation of connective tissues and meat residues from animal bones) and carrageenan on three levels 0.5%, 1.5% and 2.5%. (15 samples). The same homogenate was adulterated one of the collagen powders K4065 in weight ratios of 0.5–5% (again with increments of 0.5, additional 7 samples. Remaining four homogenates were adulterated with one of the collagens K4065 on three levels (0.5, 1.5 and 2.5%) for intraspecies variability assessment (12 samples). For repeatability study, additional 5 samples from one homogenate adulterated with 2.5% collagen K4065 were prepared. The reason for different middle level is that the chicken meat is more susceptible to adulteration with higher levels of protein, especially with collagen, which is commonly detected adulterant by state authorities. As with pork samples, 2 replicates of each homogenate were used as non-adulterated samples. In total 49 chicken samples were produced.

2.3. Sample preparation

To ensure only fine meat would be used, majority of the fat tissue was cut out from the meat chunks. The amount of fine meat used for sample production varied. Most of the pork neck chunks weighed approx. 700 g, the total amount of chicken breasts used was approximately 500 g (more breasts from the same producer had to be combined to create one coarse homogenate). In the next step 50 g of the coarse homogenate and the corresponding amount of bulking agent (the'adulterant´) were mixed and then ground again to incorporate the powder into the ground meat obtaining fine homogenate with texture similar to sausage. Approximately 45 g of the resulting meat homogenate was stored in 50 mL cuvette in a freezer at −80 °C freezer until approx. 2 h before measurement. When thawed, the meat homogenate was moulded to form a burger of dimensions approx. 5 × 5 × 0.5 cm.

2.4. Preparation of QC sample

The quality control sample was prepared by the same process as described previously, the amount of adulterant was set to 2.5% and the QC sample was prepared in 6 replicates and measured throughout the whole set of samples. These 6 samples were also used for assessment of the repeatability.

2.5. Instrumental conditions

The instrumental set-up used for measurements performed in this study was similar to that one employed earlier for detecting fish fraud (Black et al., 2017). Waters REIMS (Waters, UK) ion source was used in combination with Xevo G2-XS, a quadrupole time of flight (QTOF) mass spectrometer (Waters, UK). The mass spectrometer was operated in ´sensitivity' mode, the data were acquired both in positive or negative polarity, in mass range of m/z 100–1200 with a scan time of 0.5 s. The entrance potential was set to 40 V, heated coil potential to 80 V. The REIMS source was connected through tubing to monopolar electrosurgical knife (Model PS01-63H, Hangzhou MedStar technology Co, Ltd, Jiaxing City, China) for which power was supplied by Erbe VIO 50C generator (Erbe Medical UK Ltd, UK) with the cutting of the sample performed in “auto cut” mode of the generator with a power of 30 W. To perform mass calibration 5 mM sodium formate solution in 90% 2-propanol was used. To correct for accurate mass, 2 ng/μL Leucine-Enkephalin solution in 2-propanol (m/z 554.2615) was continuously supplied into the REIMS source by Acquity UPLC I-class system (Waters, USA) at flow rate of 0,1 mL/min. The reference solution was supplied into the REIMS source at least 30 min before measurement in order to stabilize the system. The meat homogenate was cut at least 10 times to ensure representative sampling was made from the entire surface of the

tested homogenate.

2.6. Repeatability assessment

The repeatability was determined as the variation in signal profiles of the aliquots of the same quality control (QC) sample (containing 2.5% of adulterant, PS80 protein in pork samples, collagen K4065 in chicken samples) measured 6 times throughout the sequence. In total, profiles of 73 cuts for pork and 74 cuts for chicken were used to calculate the repeatability as signal RSD and served as QC.

2.7. Multivariate analysis of REIMS data

The generated data were processed by Abstract model builder (AMX) software (Waters Research Centre (WRC), Budapest, Hungary). Mass spectra from the entire duration of the cut (approx. 6–15 spectra) were combined to produce an average spectrum of the whole cut and these spectra were binned with the step of 0.5 Da and then used to produce the statistical models. This way, more spectra represented one particular sample. The AMX software made correction for accurate mass by LeuEnk of *m/z* 554.2615 in negative mode and fragment of triacylglycerol containing oleic and palmitic acid at *m/z* 577.5196 (produced by neutral loss of one of the fatty acids), subtracted background spectra and normalized them before producing a statistical model. In our approach, model package available within AMX was not used, instead of that, the data matrix was directly exported into SIMCA (Umetrics, Sweden) for broader processing options.

For statistical models building, the data were pareto scaled and log transformed and then principal component analysis (PCA) was performed to get an overview of the data structure. Then series of partial least square and orthogonal partial least squares discriminant analysis (PLS-DA and OPLS-DA) models were built to discriminate the adulterated and non-adulterated samples and assess the lowest level of adulterant REIMS technique can detect.

3. Results and discussion

As outlined in the introduction, the use of various adulterants/undeclared additives in chicken or pork meat have been reported frequently. For the purpose of development of a high throughput screening method, several demonstration experiments were proposed. Moreover, we also tested carrageenan (sulphated polysaccharide) as it is commonly used to improve the texture of low-grade meat products but may be prohibited from use in higher quality goods. The tissues used in this study were of high quality. In the case of using different animal tissues and/or their mixes to prepare minced meats the situation may be more complicated.

The data analysis strategy focused on finding `unusual' REIMS mass spectral profiles of unadulterated meat rather than detection of the adulterants *per se*. This way, one model could be created regardless of the adulterant, because the answer of the model would be `compliant' or `non-compliant`. Using such a binary model strategy, if the sample would be classified as `non-compliant`, a series of alternative models could be employed to discover the nature of the adulterant or additional methods such as LC-MS could be employed to investigate the `suspect' sample further. R^2 (cumulative), Q^2 (cumulative) and Root Mean Squared Error of cross validation (RMSECV) were used to determine the validity of the models. R^2 (cum) indicates the variation described by all components in the model and Q^2 (cum) is a measure of how accurately the model can predict class membership.

3.1. Pork samples analysis

Samples of pork neck homogenate were mixed with a wide range of additives. These were PS80 pork protein in three variants (80, 85, and 90) according to their average protein content, Protein Natur – hydrolysed pork protein powder, pork plasma powder and carrageenan.

One of the problems experienced during REIMS based measurements was rather poor repeatability of generated signal intensities as the source appeared to be getting clogged by the solid particles generated at the sampling site after several hours of cutting. Concerning the repeatability of the analysis, 6 burgers prepared from the same meat/adulterant mix were analysed throughout the sequence of 46 or 49 samples. Repeatability was calculated as the range of relative standard deviations of peak intensities within the mass spectra of the same sample. The range of relative standard deviations for ion intensities in pork samples was 6.8%–77.1%, with a median of 25.1% (1st quartile 22.2%, 3rd quartile: 28.2%). This indicates that REIMS is a reasonably sensitive procedure but can struggle in terms of repeatability, however it can make up to this disadvantage with its very fast response time.

The models built in SIMCA were focused on revealing unusual patterns in the REIMS spectra of meat samples rather than grouping them into classes by adulterant type and/or weight ratio. The latter strategy would overtrain the statistical model. Even using one group for each adulterant would produce models with low predictive ability, thus binary models were favoured as the main concern is if the sample is conforming or not. Therefore, both PLS-DA and OPLS-DA models were used. To asses the detectability of the method, samples on a level of interest (0.5, 1.0 and 2.5%) and higher were used for model building. The R^2 (cum) and Q^2 (cum) values of the models were checked to assess the ability to spot unusual profiles of REIMS mass spectra of the meat samples. Both spectra obtained in positive and negative modes of ionization were tested. Better data was obtained in negative mode. Two regions of the mass spectra were also tested: low mass region of *m/z* 100–500 which contains mainly fatty acid fragments generated from phospholipids and high m/z region of 600–950 which is used in most of

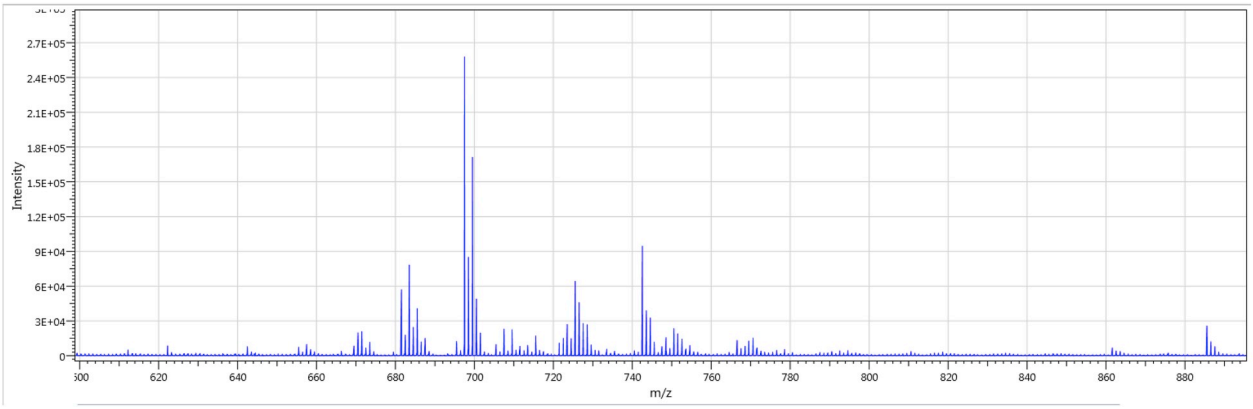

Fig. 1. Average REIMS- spectrum in the region of *m/z* 600–900 of unadulterated chicken sample containing negatively charged ions of anionic phospholipids.

Table 1
Results of cross-validation of PLS and OPLS-DA statistical models in two different mass ranges and polarities built to differentiate adulterated and non-adulterated pork samples.

REIMS- 100–500 m/z								
% of adulterant in meat	PLS-DA				OPLS-DA			
	no. of components	R^2 (cum)	Q^2 (cum)	RMSECV	no. of components	R^2 (cum)	Q^2 (cum)	RMSECV
0.5	11	84.5%	65.6%	23.1%	1 + 4+0	58.5%	48.5%	27.3%
1	10	88.4%	71.9%	22.0%	1 + 5+0	71.5%	59.0%	26.6%
2.5	10	90.8%	75.7%	21.9%	1 + 5+0	75.5%	63.4%	26.0%
REIMS- 600–950 m/z								
% of adulterant in meat	PLS-DA				OPLS-DA			
	no. of components	R^2 (cum)	Q^2 (cum)	RMSECV	no. of components	R^2 (cum)	Q^2 (cum)	RMSECV
0.5	12	86.30%	71.10%	21.20%	1 + 8+0	79.50%	62.40%	23.30%
1	11	89.70%	76.30%	20.70%	1 + 6+0	80.80%	67.30%	23.80%
2.5	9	94.80%	90.00%	14.90%	1 + 5+0	89.50%	85.20%	17.70%
REIMS+ 100–350 m/z								
% of adulterant in meat	PLS-DA				OPLS-DA			
	no. of components	R^2 (cum)	Q^2 (cum)	RMSECV	no. of components	R^2 (cum)	Q^2 (cum)	RMSECV
0.5	6	68.00%	50.00%	28.30%	1 + 4+0	56.70%	35.10%	32.30%
1	6	73.50%	56.20%	28.30%	1 + 4+0	63.10%	43.10%	32.40%
2.5	6	83.90%	70.20%	25.80%	1 + 5+0	83.90%	55.20%	31.60%
REIMS+ 500–950 m/z								
% of adulterant in meat	PLS-DA				OPLS-DA			
	no. of components	R^2 (cum)	Q^2 (cum)	RMSECV	no. of components	R^2 (cum)	Q^2 (cum)	RMSECV
0.5	8	86.90%	63.60%	24.10%	1 + 3+0	57.70%	41.80%	30.50%
1	9	92.10%	72.30%	22.60%	1 + 3+0	65.80%	46.80%	31.30%
2.5	10	97.40%	83.20%	19.30%	1 + 5+0	87.80%	66.30%	27.30%

the published REIMS studies concerned with tissues or cell cultures and is dominated by anionic phospholipids in the negative mode and triacylglycerols in positive mode, the example of such spectrum in negative mode is presented in Fig. 1. The reason for using these mass ranges was that these are the regions where almost all of the ions in the spectra are. The ranges are correlated as the lower mass range contains fragments of the higher mass ions. However, a single fragment may come from different high mass precursors which may or may not be useful for the statistical modelling thus the lower range was employed to assess this possibility. The data were log transformed and pareto scaled. In general, profiles of triacylglycerols and anionic phospholipids in high m/z regions (as identified by METLIN online library) were more reliable than profiles of their fragments (in low mass m/z region). The results of SIMCA internal cross-validation of built models are summarized in Table 1. The PLS-DA models based on a high mass region in negative mode have the highest values of R^2(cum) and Q^2(cum) as well as the lowest value for RMSECV. The performance of OPLS-DA models was surprisingly lower than of those non-orthogonal models. For the model to be used in practice, high prediction ability (high Q^2(cum) is required) is required, however, most of the Q^2 (cum) values were below 90% which would be enough to classify the sample as non-conforming or "unusual" reliably. The only model exceeding 90% limit was PLS-DA model where the level of adulterant was at least 2.5%, its score plot is shown in Fig. 2. The only exception was carrageenan adulterated pork which was discernible even at the 0.5% level and clearly different at 1.0% of carrageenan content.

The most prominent variables could be obtained from corresponding loadings plot or variable importance in projection (VIP) plots. We used the latter plot for important variable evaluation. In the VIP plot, the variables are ordered according to their VIP scores. Variables which have the highest effect on class separation have the highest VIP score which is usually more than 1. For the final model (2.5% of the adulterants), the 'enhanced' variables in adulterated samples were *m/z* 764.75, 762.75, 738.75, 704.75 and 719.75. The important 'suppressed' variables were 718.75 and 717.75 (isotopic peak). However, it can be concluded that the 'suppressed' variables were less important than the those upregulated as the decrease, when investigated visually, was less consistent and they also exhibited lower VIP score values. For the 'enhanced' variables there was a clear increase in the previously mentioned variables as illustrated on the trend plot of ion 762.75 in Fig. 3. The intensities of the ion may overlap in some samples, thus indicating that this variable would not suffice for group discrimination on its own. However, the response of the model is multivariate and multiple significant variables play a role, thus making the model successful. The statistical model was validated by a permutation test with 100 permutations, the intercepts were R2 = (0.0,0.67), Q2 = 0.0,0.0187). The plot for the permutation test is presented in Supplemental Fig. 1 and suggests good performance of the model. It is worth to notice, that although the significant ions could be tentatively identified by METLIN database, in this case as phospholipids, the nature of the signals is not important for the model.

Consequently, if a sample is classified as "non-conforming", by the previously described model, it should be investigated further. This could be achieved either by inserting it into another model to classify the type of adulterant or by employing a different technique to reveal more information about the sample. Following the first model, additional model was built and employed. This model could discover if the "non-conforming" sample exhibits profiles similar to that of known adulterants. This model had R^2(cum) = 95.1 %and Q^2(cum) = 84.0%. Some adulterants such as Protein Natur and pork plasma were found to exhibit very similar profiles. This indicates that it may be hard to distinguish them, however, REIMS is meant as a fast first-step screening method and not as in-depth investigative tool.

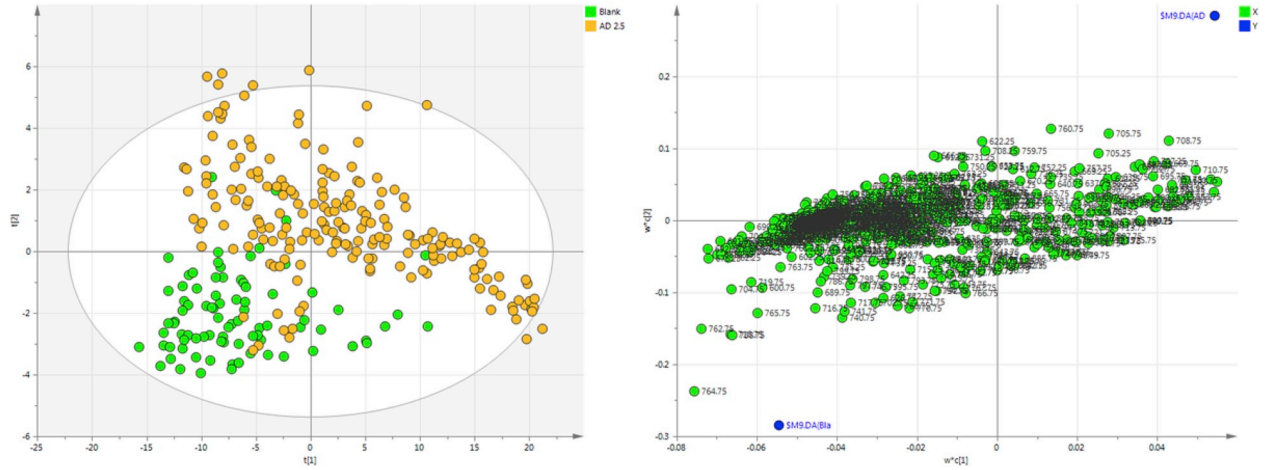

Fig. 2. PLS-DA score plot and its corresponding loadings plot for pork samples showing fair discrimination between unadulterated samples and samples adulterated with 2.5% of adulterants (w/w).

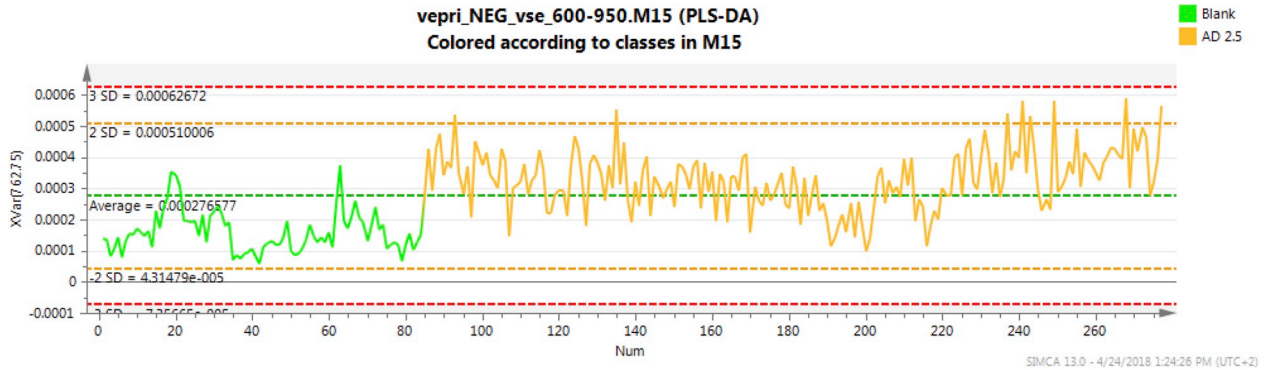

Fig. 3. Trend plot of the ion 762.75 showing its abundance in non-adulterated pork samples (green line) and samples adulterated with at least 2.5% of bulking agent (orange line), x axis shows the sample numbers. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

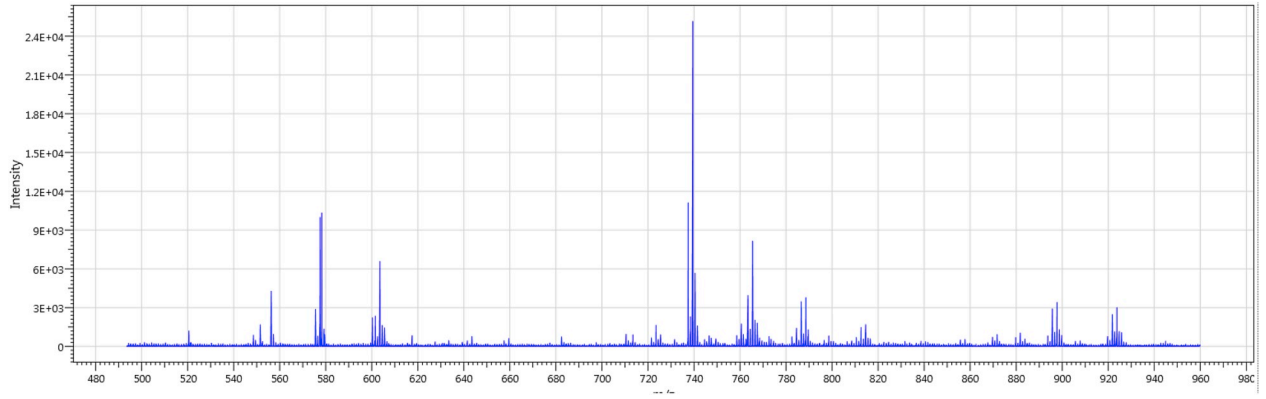

Fig. 4. Average REIMS + spectrum in the range of m/z 600–950 of a non-adulterated chicken sample containing mainly triacylglycerols and their fragments (neutral loss of fatty acyl).

3.2. Chicken sample analysis

The set of bulking agents used to "adulterate" chicken samples was different than that used for pork samples. The reason is that these bulking agents are more commonly used to alter the properties of chicken meat and most of them are based on collagen. They are usually used for water binding and altering the texture of the final product. The strategy was identical to that of pork samples. In the search for "typical" chicken meat profiles, we measured homogenate without additives and then compared them to the adulterated samples. To estimate the detectability of the method we built models with the level of adulterant 0.5% and higher, 1.5% and higher and finally 2.5% and higher until reaching the required model performance of Q2 = 90%. The maximum amount of additive was chosen to reflect amounts used in practice when the additives are declared. The middle level of adulterant was 1.5%, which was different from pork samples. The rationale behind this is that higher amounts of bulking agents are generally added to chicken meat. A 'calibration curve' was also constructed for one of the adulterants to determine if there was a concentration dependent drift in the models.

A set of statistical models was produced in order to recognize

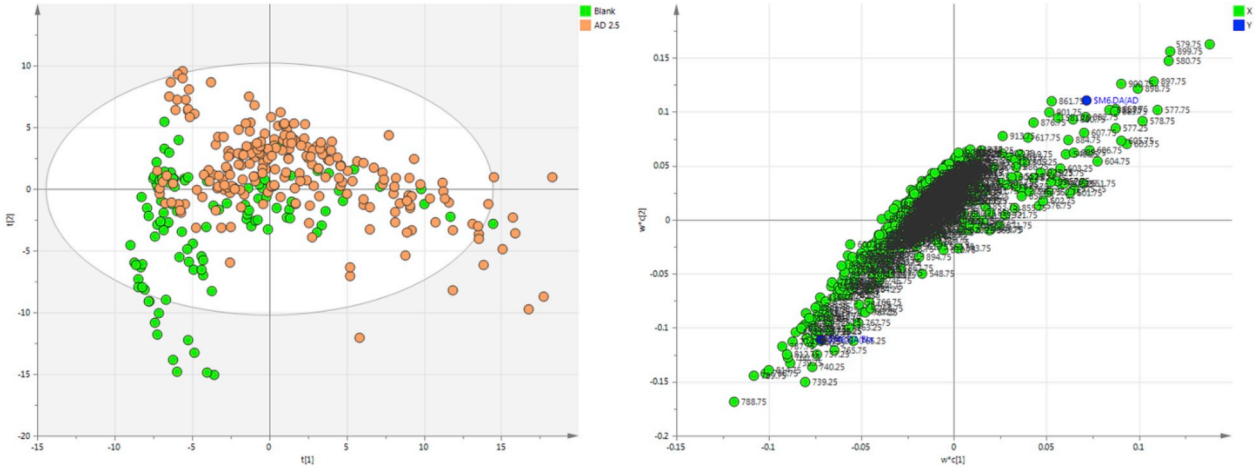

Fig. 5. PLS-DA model score plot and corresponding loadings plot. Model allows discrimination of adulterated and non-adulterated chicken samples on the lowest level of 2.5 percent. Non-adulterated samples are in green, adulterated samples in orange. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Results of cross-validation of PLS and OPLS-DA statistical models in two different mass ranges and polarities built to differentiate adulterated and non-adulterated chicken samples.

REIMS- 100–450 m/z								
% of adulterant in meat	PLS-DA				OPLS-DA			
	no. of components	R^2 (cum)	Q^2 (cum)	RMSECV	no. of components	R^2 (cum)	Q^2 (cum)	RMSECV
0.5	7	65.6%	52.1%	27.6%	1 + 5+0	60.7%	44.9%	29.6%
1	7	72.0%	57.5%	27.8%	1 + 5+0	68.3%	52.5%	29.3%
2.5	8	83.7%	66.9%	28.7%	1 + 5+0	74.1%	59.9%	29.8%
REIMS- 600–950 m/z								
% of adulterant in meat	PLS-DA				OPLS-DA			
	no. of components	R^2 (cum)	Q^2 (cum)	RMSECV	no. of components	R^2 (cum)	Q^2 (cum)	RMSECV
0.5	18	89.7%	69.6%	21.6%	1 + 9+0	69.8%	55.0%	26.8%
1	14	87.9%	76.6%	21.8%	1 + 9+0	77.8%	66.6%	24.6%
2.5	14	92.7%	84.1%	20.1%	1 + 9+0	84.5%	73.8%	23.9%
REIMS+ 100–350 m/z								
% of adulterant in meat	PLS-DA				OPLS-DA			
	no. of components	R^2 (cum)	Q^2 (cum)	RMSECV	no. of components	R^2 (cum)	Q^2 (cum)	RMSECV
0.5	7	64.8%	46.5%	28.8%	1 + 4+0	50.7%	36.1%	31.7%
1	8	82.2%	64.0%	25.0%	1 + 4+0	64.6%	52.3%	29.1%
2.5	8	91.7%	79.2%	21.5%	1 + 4+0	81.3%	69.5%	25.9%
REIMS+ 600–950 m/z								
% of adulterant in meat	PLS-DA				OPLS-DA			
	no. of components	R^2 (cum)	Q^2 (cum)	RMSECV	no. of components	R^2 (cum)	Q^2 (cum)	RMSECV
0.5	8	77.3%	57.9%	25.4%	1 + 5+0	63.6%	42.2%	30.1%
1	8	90.1%	78.0%	20.2%	1 + 5+0	80.3%	66.6%	25.0%
2.5	9	97.1%	87.8%	17.5%	1 + 4+0	88.3%	77.8%	22.2%

adulterated samples with an increasing content of adulterant. The values for predicted variance were generally lower than those for pork. One of the possible reasons for this is a higher variance in chicken meat composition because as diverse types of chicken were included as possible, thus mimicking a real situation. Surprisingly, the model based on high-mass region (containing triacylglycerols and triacylglycerol fragments as can be seen in Fig. 4) in positive mode performed better than high-mass region in negative mode with R^2 (cum) = 97.1% and Q^2 (cum) = 87.8% for the model including blank samples and samples with 2.5% of adulterant and higher. The repeatability of the spectra was similar to that of pork samples. It ranged from 11.1% to 75.1% with median of 28.1% (1st quartile 24.4%, 3rd quartile: 32.0%). The separation of groups of samples was not obvious when looking at PLS-DA plots such as the one in Fig. 5, however, the process of discrimination takes place in other dimensions as well. The most prominent signals 899.75 and 897.75 belonged to those of triacylglycerols (tentatively identified by METLIN database). It is again worth noting that the response is multivariate and none of these variables would have such a descriptive value when used alone. The model was validated by SIMCA internal cross-validation, its results are presented in Table 2. Permutation test was also performed for the model validation, the intercepts from the permutation test (with 100 permutations) were: R2 = (0.0,0.89), Q2 = (0.0,0.436), the plot can be seen in Supplemental Fig. 2. Although the Q2 intercept is high above zero on Y axis, the plot

suggests that the model is valid.

4. Conclusions

In this study, it was shown that REIMS in combination with multivariate analysis can be a very promising fast analytical tool to discover unusual patterns in pork and chicken meat which can point to adulteration with (mainly) protein based bulking agents. The lowest level of adulterant the strategy is able to reliably identify is 2.5% for protein-based additives, carrageenan could be discovered at level as low as 1.0%. The variability in spectra of one sample seems to be high (median RSD for ions 25.1% for pork (N = 73) and 28.1% for chicken samples (N = 74), however the measurement of the sample takes just a few seconds and its evaluation another few minutes or, when the PLS-DA model is implemented into recognition software a few seconds. REIMS is therefore an ideal tool for high throughput analysis of a high number of samples identifying ´suspects 'which require further examination by additional methods such as PCR or LC-MS.

Declaration of interest

None.

Acknowledgments

This research was supported by the European Union´ s Horizon 2020 research and innovation programme under grant agreement No 692195 (MultiCoop) and by the Czech Republic National Agency for Agricultural Research (Project no. QJ1530272). This work was also supported by the “Operational Programme Prague – Competitiveness” (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503) and the “National Programme of Sustainability I” - NPU I (LO1601- No.: MSMT-43760/2015).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2018.10.029.

References

Abbas, O., Zadravec, M., Baeten, V., Mikuš, T., Lešić, T., Vulić, A., et al. (2018). Analytical methods used for the authentication of food of animal origin. *Food Chemistry, 246*, 6–17. https://doi.org/10.1016/j.foodchem.2017.11.007.

Balog, J., Perenyi, D., Guallar-Hoyas, C., Egri, A., Pringle, S. D., Stead, S., et al. (2016). Identification of the species of origin for meat products by rapid evaporative ionization mass spectrometry. *Journal of Agricultural and Food Chemistry, 64*(23), 4793–4800. https://doi.org/10.1021/acs.jafc.6b01041.

Black, C., Chevallier, O. P., & Elliott, C. T. (2016). The current and potential applications of Ambient Mass Spectrometry in detecting food fraud. *TRAC Trends in Analytical Chemistry, 82*, 268–278. https://doi.org/10.1016/j.trac.2016.06.005.

Black, C., Chevallier, O. P., Haughey, S. A., Balog, J., Stead, S., Pringle, S. D., et al. (2017). A real time metabolomic profiling approach to detecting fish fraud using rapid evaporative ionisation mass spectrometry. *Metabolomics, 13*(12)https://doi.org/10.1007/s11306-017-1291-y.

Hajslova, J., Cajka, T., & Vaclavik, L. (2011). Challenging applications offered by direct analysis in real time (DART) in food-quality and safety analysis. *TRAC Trends in Analytical Chemistry, 30*(2), 204–218. https://doi.org/10.1016/j.trac.2010.11.001.

Leitner, A., Castro-Rubio, F., Marina, M. L., & Lindner, W. (2006). Identification of marker proteins for the adulteration of meat products with soybean proteins by multidimensional liquid Chromatography – Tandem mass spectrometry. *Journal of Proteome Research, 5*(9), 2424–2430. https://doi.org/10.1021/pr060145q.

Takats, Z., Denes, J., & Kinross, J. (2012). Identifying the margin: A new method to distinguish between cancerous and noncancerous tissue during surgery. *Future Oncology, 8*(2), 113–116. https://doi.org/10.2217/fon.11.151.